REMARKS

Status of the Claims

Claims 1-49 are currently pending and under consideration in the present application. Claims 1-49 are currently rejected by the Examiner.

Rejections Under 35 U.S.C. § 112, Second Paragraph

The Examiner states that Claims 41, 46 and 47 are rejected under 35 U.S.C., second paragraph, "as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention" (See, Office Action Pg. 2).

Specifically, the Examiner states that claim 41 is "vague and indefinite in the recitation of 'also overexpressed'" (see, Office Action Pg. 2). Per the examiner's request and to expedite prosecution of the instant application, claim 41 has been amended to remove the word "also."

Claims 46 and 47 are rejected as the Examiner states that recitation of "cancer cell" lacks antecedent basis in claim 40. Claim 40 has been amended to specifically recite a cancer cell, creating appropriate antecedent basis for the term "cancer cell" in claims 46 and 47.

As the claims have been appropriately amended to address the Examiner's rejections, Applicants respectfully request withdrawal of the rejections under 35 U.S.C. § 112, second paragraph.

Rejections Under 35 U.S.C. § 112, First Paragraph

The Examiner states that Claims 24-29 and 31-39 are rejected under 35 U.S.C. § 112, first paragraph, as the specification is "enabling for a method of treating cancer cells, but not enabling for methods of treating non-cancer cells (see, Office Action Pgs. 2-3). In order to address these rejections and expedite prosecution, Claim 24 has been amended to be drawn to a "cancer cell"; thus claims 24-29 and 31-39 now all read to methods directed towards cancer cells.

As the claims have been appropriately amended to address the Examiner's rejections, Applicant's respectfully request withdrawal of the rejections under 35 U.S.C. § 112, first paragraph.

Rejections Under 35 U.S.C. § 102(b)

The Examiner rejects Claim 49 under 35 U.S.C. 102(b) as being anticipated by Benatti et al. (WO 96/01893) (see, Office Action Pgs. 3-4). The Examiner states that claim 49 is drawn in part to an isolated nucleic acid sequence (SEQ ID NO:14) that is identical to SEQ ID NO:12 disclosed in Bernatti, et al. In order to address the rejection and facilitate prosecution, claim 49 has been amended to not include SEQ ID NO:14.

As the claim has been appropriately amended to address the Examiner's rejection, Applicant's respectfully request withdrawal of the rejection under 35 U.S.C. § 102(b).

Rejections Under 35 U.S.C. § 103(a)

As set forth in M.P.E.P. § 2141 (I), the Patent Office's policy is to follow *Graham v. John Deere Co. of Kansas City*, 383 U.S. 1 (1966), in the consideration and determination of obviousness under 35 U.S.C. § 103(a). The four factual inquires enunciated in *Graham* for determining obviousness are as follows:

(A) Determining the scope and contents of the prior art;

(B) Ascertaining the differences between the prior art and the claims at issue;

(C) Resolving the level of ordinary skill in the pertinent art; and

(D) Evaluating evidence of secondary considerations.

Recently, the U.S. Supreme Court affirmed the holding of *Graham* regarding obviousness. *See, KSR Int'l Co. v. Teleflex Inc.*, 127 S.Ct. 1727 (2007).

To establish a *prima facie* case of obviousness, three basic criteria must be met: (1) there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings; (2) there must be a reasonable expectation of success; and (3) the prior art references must teach or suggest all the claim limitations. *See,* M.P.E.P. § 2143.

Among the secondary considerations specifically discussed in *KSR*, the Court emphasized surprising or unexpected results as being indicative of non-obviousness. As such, obviousness is rebuttable by the Applicants' showing of secondary considerations in the form of unexpected results. Applicants assert that the results with the diphtheria toxin fusion proteins of the present invention were in fact unexpected.

A. Leppla, et al.,(WO 01/21656) in view of Leppla, et al., (US 5,677,274) and Frankel, et al.

Claims 1-6, 8, 11, 12, 17, 20-22, 24-30, 32, 34, 35 and 40-47 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Leppla, et al., (WO 01/21656) in view of Leppla, et al., (US 5,677,274) and Frankel, et al., (see, Office Action Pg. 4).

The Examiner states that Leppla, et al., (WO 01/216565) "teaches a method of targeting a cell overexpressing matrix metalloproteinase or plasminogen activator, wherein said cells include cancer cells" and "comprising administering to said cells a recombinant anthrax protein wherein said protein comprises matrix metalloproteinase cleavage sites" or "plasminogen activator cleavage sites in place of the native furin-cleavage site. Further Leppla, et al., teaches an "embodiment wherein said recombinant protein comprises a heterologous receptor binding domain which is a growth factor" and the said recombinant protein kills tumor cells. (See, Office Action Pg. 6.)

The Examiner states that Leppla, et al., (US 5,677,274) teaches "that any specific protease site can be introduced into any natural or recombinant toxin, including diphtheria toxin." (See, Office Action, Pg. 7).

The Examiner also states that Frankel, et al., teaches that "DT comprises an N-terminal catalytic domain, a furin-sensitive loop and a translocation domain (amino acids 1-388) followed by a cell-binding domain (amino acids 389-535)." According to the Examiner, Frankel, et al., further teaches that "the cell binding domain of DT binds to heparin-binding epidermal growth factor-like growth factor" and that one "of skill in the art would reasonable ascertain that the cell binding domain of native DT did not exert cell-type specificity." The Examiner further states that Frankel, et al., teaches "the fusion of the catalytic and translocation domains of DT

without the cell binding domains of DT fused to u-PA, but notes the damage to human endothelial cell in vitro" and that said fusion protein "retain the native furin site." (See, Office Action, Pg. 7.)

The Examiner states that it "would have been prima facie obvious to one of skill in the art at the time the invention was made to substitute residues 1-388 of DT, wherein said DT had been recombinantly engineered in a similar manner as the anthrax toxin to replace the furin cleavage site with matrix metalloproteinase cleavage sites, or plasminogen activator cleavage sites, wherein the recombinant DT was also a fusion protein with a growth factor, for binding to cells that over express said growth factor." (See, Office Action Pg. 7)

B. Leppla, et al., (WO 01/21656) in view of Leppla, et al., (US 5,677,274) and Frankel, et al., in further view of Frankel, et al. (2002), and Scherrer, et al.

Claims 1-13, 16-36, and 39-48 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Leppla, et al., (WO 01/21656), Leppla, et al., (US 5,677,274), and Frankel, et al., as applied to claims 1-6, 8, 11, 12, 17, 20-22, 24-30, 32, 34, 35 and 40-47, in further view of Frankel, et al., and Scherrer, et al. (See, Office Action Pg. 7.)

The Examiner states that Frankel, et al.(2002), teaches "the administration of a DT fusion protein wherein the heterologous protein is GM-CSF." The Examiner further states that Frankel, et al., teaches that "the DT fusion protein provided a low level of clinical activity in patients with chemoresistant AML" and suggests "that liver toxicity was due to the GM-CSF binding Kuffer cells." Further, the Examiner states that the abstract of Scherrer, et al., teaches that leukemic cells from patients with AML exhibited high enzymatic activity for u-PA." (See, Office Action Pg. 9.)

The Examiner further states that it "would have been prima facie obvious to provide the nucleic acid encoding the engineered DT protein rendered obvious by the combination of Leppla et al (WO 01/21656), Leppla et al (U.S. 5,677,274) and Frankel et al wherein the furin cleavage sites were replaced by u-PA cleavage sites and wherein the heterologous protein was GM-CSF." (See, Office Action Pg. 9.)

C. Leppla, et al., (WO 01/21656) in view of Leppla, et al., (US 5,677,274) and Frankel, et al., in further view of Faller, et al., (WO 95/11699)

Claims 1-12, 14, 17, 20-22, 24-35, 37, 40-47 and 49 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Leppla, et al., (WO 01/21656), Leppla, et al., (US 5,677,274) and Frankel, et al., as applied to claims 1-6, 8, 11, 12, 17, 20-22, 24-30, 32, 34, 35 and 40-47 in further view of Faller, et al., (WO 95/11699) (see, Office Action Pgs. 9-10).

The Examiner states that Faller, et al., teaches "the treatment of leukemia cells with a DAB389-Il-2 fusion toxin, wherein DAB389 comprises residues 1-189 of DT." (See, Office Action, Pg. 10)

The Examiner also states that it "would have been prima facie obvious at the time that the claimed invention was made to use Il-2 as the heterologous receptor binding protein of the engineered DT-growth factor fusion protein" and that one "of skill in the art would have been motivate[e]d to do so by the teachings of Faller et al on the treatment of leukemias by administration of the DT-Il-2 fusion protein." (See, Office Action, Pg. 10)

D. Leppla, et al., (WO 01/21656) in view of Leppla, et al., (US 5,677,274) and Frankel, et al., in further view of Heimbrook, et al.

Claims 1-6, 8-12, 15, 17, 20-22, 24-30, 32-35, 38, 40-47 and 49 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Leppla, et al., (WO 01/21656), Leppla, et al., (US 5,677,274) and Frankel, et al., as applied to claims 1-6, 8, 11, 12, 17, 20-22, 24-30, 32, 34, 35 and 40-47 and in further view of Heimbrook, et al., (see, Office Action Pg. 10).

The Examiner states that Heimbrook, et al., teaches "fusion proteins with toxins, wherein the heterologous polypeptide is TGF-alpha which is a ligand for the EGF receptor" and "that tumors possess EGFR and some tumor types exhibit increased numbers of EGFR relative to normal tissue" (see, Office Action pgs. 10-11).

The Examiner also states that it "would have been prima facie obvious at the time that the claimed invention was made to provide for an engineered DT fusion protein which comprises as the heterologous protein, EGF for binding to the EGFR" and that one "of skill in

the art would have been motivated to do so by the analogous example taught" by Heimbrook, et al., (see, Office Action, Pg. 11).

E. Unexpected Results Over the Prior Art

Applicants assert that one of ordinary skill in the art would **not** have had a reasonable expectation of success by incorporating matrix metalloproteinase or plasminogen activator-protease cleavage sites in place of the native furin cleavage site as taught by Leppla, et al., (WO 01/21656) in view of the teachings of Leppla, et al., (5,677,274) regarding the substitution of any specific protease site within a natural recombinant toxin and that the results obtained were unexpected. Applicants further assert in view of Leppla, et al., (WO 01/21656), Frankel, et al. (2002), Scherrer, et al., Faller, et al., (WO 95/11699), and Heimbrook, et al., that the present invention is still **not** obvious as the results embodied by the present invention are still unexpected even in view of the additional references.

Diphtheria toxins, such as those of the present invention, are activated intracellularly, either by bacterial proteases during secretion processes or within the endosomes of target cells. This is in contrast to metalloproteinases, plasminogen activator and anthrax toxins, all of which are activated on the surface of target cells. As such, it is unexpected that diphtheria toxin would be able to become activated on the surface of target cells. (See, e.g. Faller, et al., Heimbrook, et al., or Leppla, et al., WO 01/21656).)

With regard to the anthrax toxins taught by Leppla, et al., (WO 01/21656), anthrax toxins are typically cleaved extracellularly or at the surface of target cells. It is unexpected that incorporating matrix metalloproteinase or plasminogen activator cleavage sites in place of the native furin cleavage site as taught by Leppla, et al., (WO 01/21656) in view of the teachings of Leppla, et al., (5,677,274) regarding the substitution of any specific protease site within a natural recombinant toxin would allow for the extracellular cleavage and activation of the diphtheria toxins as taught by the present invention. In contrast to the anthrax toxins taught by Leppla, et al., (WO 01/21656) which are activated extracellularly or at the surface of the target cell, the native diphtheria toxins are activated intracellularly. Due to the very different locations where diphtheria toxins and anthrax toxins are normally activated, it is unexpected that

diphtheria toxins even with substitution of the native furin cleavage site would be able to be activated extracellularly or on the surface of the target cell, as the present invention discloses.

With regard to the GM-CSF fusions taught by Frankel, et al. (2002), in which the furin cleavage sites were replaced by u-PA cleavage sites, these fusions function after "receptor-mediated endocytosis, the fusion protein reaches the early endosomes, to which it is cleaved by furin" and "inserts into the vesicle membrane and facilitates the escape of the DT-A fragment into the cytosol," thus leading to cell death (see, Frankel, et al. (2002), Pg. 1005, column 1, paragraph 1). In the methods taught by Frankel, et al. (2002), the DT fusions are cleaved and activated intracellularly, functioning only after intracellular cleavage.

It is unexpected that diphtheria toxin fusions of the instant invention would undergo extracellular cleavage and activation rather than requiring intracellular cleavage in a manner similar to that of the DT-GM-CSF fusions of Frankel, et al. (2002), wherein activation occurred in the endosome. Combining Frankel, et al. (2002), describing intracellular cleavage and activation of a DT-GM-CSF fusion with Leppla, et al.,(WO 01/21656), Leppla, et al., (US 5,677,274), and Frankel, et al., describing substitution of the native intracellular furin cleavage site with an extracellular cleavage site that is either a matrix metalloproteinase cleavage site or a plasminogen activator cleavage site would not provide for a method with a reasonable expectation of success, and thus the results are unexpected regarding extracellular cleavage of diphtheria toxin fusions.

Additionally, in response to the Examiner's reference to the abstract of Scherrer, et al., and that it teaches that leukemic cells from patients with AML exhibited high enzymatic activity for u-PA, this reference provides no basis for obviousness. As it is unexpected that the diphtheria toxins can be cleaved extracellularly, it is unexpected that extracellular u-PA can cleave the diphtheria toxins. Accordingly, reference to extracellular proteases and the discussion of u-PA secretion by AML is no longer relevant to the issue of obviousness.

With regard to the DAB389 IL2-R peptides taught by Faller, et al., said methods employ peptide fusions of diphtheria toxin (DT) residues 1-486 or 1-389 with IL-2. Faller, et al., teaches methods for generating recombinant peptides "capable of selectively targeting and killing high-affinity IL-2R bearing cells. When presented to cells with high affinity IL-2

receptors, the recombinant protein undergoes IL-2R-specific binding and internalization via receptor-mediated endocytosis. Processing occurs within the acidic endosome, and the A fragment of the toxin passes into the cytosol" and results "in cell death." (See, pg. 11, first full paragraph, continued on pg. 12.) The fusion proteins taught by Faller, et al., are cleaved and activated intracellularly. Again it is unexpected that diphtheria toxin fusions of the instant invention would undergo extracellular cleavage and activation rather than requiring intracellular or endosomal cleavage in a similar manner to the DAB389 IL2-R fusions of Faller, et al.

With regard to the TGF-alpha-pseudomonas exotoxin fusions taught by Heimbrook, et al., pseudomonas exotoxins are also typically cleaved intracellularly in the endosome. (See, e.g., Chiron, et al., *Furin-mediated Cleavage of Pseudomonas Exotoxin-derived Chimeric Toxins*, Journal of Biological Chemistry, 275(50):31707-31711 (1997).) One of skill in the art would understand the peptides disclosed by the methods of Heimbrook, et al., upon binding to EGFR expressing cells, are cleaved and activated intracellularly. Again it is unexpected that diphtheria toxin fusions of the instant invention would undergo extracellular cleavage and activation rather than requiring intracellular or endosomal cleavage and activation in a similar manner to the TGF-alpha-pseudomonas exotoxin fusions of Heimbrook, et al..

Applicants assert that the methods of the present invention are directed towards diphtheria toxins and diphtheria toxin peptides capable of undergoing cleavage and activation in the extracellular environment or on the surface of a target cell. As the prior art cited by the Examiner teaches methods of intracellular or endosomal activation and cleavage of diphtheria toxins and diphtheria toxin fusions, it is unexpected that diphtheria toxins would be able to undergo cleavage and activation in non-intracellular or non-endosomal environments. As such, the methods of the present invention are unexpected in that the diphtheria toxins of the instant invention are in fact capable of undergoing cleavage and activation in the extracellular environment or on the surface of a target cell.

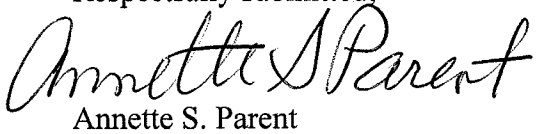
Based on the above arguments, Applicants respectfully request removal of all of the obviousness rejections under 35 U.S.C. § 103(a).

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 925.472.5000.

Respectfully submitted,



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